bill the fee required pursuant to 37 C.F.R. §1.17(a)(3) to the deposit account indicated at the conclusion of this letter. Please consider the following amendments and remarks.

IN THE SPECIFICATION

Please **amend** specification at paragraph 18 with the follow rewritten paragraph:

Q1

[00018] (AMENDED) In addition, the present invention is directed to a protein kinase inhibitor binding site which is outside the ATP binding site. This protein kinase inhibitor binding site which is outside the ATP binding site is useful, *inter alia,* for the rational design of non-ATP binding site inhibitors of protein kinases. In a preferred embodiment, the inhibitor binding site of the present invention which is outside the ATP binding site comprises an amino acid sequence corresponding to an amino acid sequence of p38 and structurally homologous to a domain of p38 wherein said domain is defined by a start point at linker L5 (residues 78-85 of SEQ ID NO:1) that joins helix C (residues 63-77 of SEQ ID NO:1) with β4 (residues 84-89 of SEQ ID NO:1), the crossover connection (L7) (residues 100-115 of SEQ ID NO:1) and an end point at the C-terminus (βL16) (residues 310-336 of SEQ ID NO:1) of p38. This domain has corresponding amino acids in IKK-β, Map/ERK kinase, eJNK, MEK, GSK-3, Akt, and NIK. *See* Figure 7. Therefore, a further embodiment of the present invention is a method of designing a protein kinase inhibitor comprising (1) employing the structure of a protein kinase bound to an inhibitor at an inhibitor binding site (wherein the inhibitor binding site may be identified previously or found by screening) (2) applying standard methods of structure-based drug design based using structure of the protein kinase bound to the inhibitor to identify and characterize additional inhibitors directed to the inhibitor binding site.

Please **amend** specification at paragraph 19 with the follow rewritten paragraph:

Q2

[00019] (AMENDED) The patent application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The present invention may be better understood with reference to the attached drawings in which—

FIGURE 1 provides a sequence alignment of p38 (SEQ ID NO:1) with (a) IKK-β (SEQ ID NO:2) and (b) MEK1 (SEQ ID NO:4). The consensus sequences in (a) and (b) correspond to SEQ ID NOS:3 and 5, respectively. In bold are the residues which are identical. The low consensus residues are underlined and the neutral residues are in normal type. p38 shares 25-30% identity with IKK-β and MEK1.

FIGURE 2 provides a chemical formula of (a) sulindac sulfide and (b) PD98059. The different moieties in the molecules as used in the text are labelled for clarity.

FIGURE 3 provides a close-up depiction of the structure of p38 bound to (a) sulindac sulfide, and (b) PD98059. Also, the dotted lines indicate the hydrogen bonds between the inhibitor molecule and p38.

FIGURE 4 provides a depiction of the structure of the inhibitor binding site of the present invention showing the binding of sulindac sulfide and PD98059 is depicted on a p38 molecule and superimposed is the well-known ATP-competitive inhibitor binding site showing the SB203580 molecule. The activation lip is shown in red.

FIGURE 5 provides a ribbon diagram of p38 indicating the numbering scheme of the helices and β-strands. The amino acid numbers of SEQ ID NO:1 corresponding to the

A2 cont.

various helices and strands in p38 are as follows:

α_c(63-77), α_d(113-119), α_e(123-143), α_f(203-218), α_g(228-238), α_h(279-289), α_l(299-304), β1(25-33), β2(36-43), β3(48-56), β4(87-91), β(101-107), β6(146-150), β7(156-159), β8(163-167), β9(173–177) and C-terminus 345-356. The sequence numbers absent in the list are those of the linkers.

FIGURE 6 provides a close-up of superposition of native p38 (blue) and Sulindac bound p38 (green) showing conformational changes in inhibitor binding site and activation loop.

FIGURE 7 provides a sequence alignment near the two inhibitor binding sites of p38 (residues 25-42, 63-77, 78-85, 100-115, and 345-356 of SEQ ID NO:1 respectively) with IKKβ (Accession AAF21978) (residues 21-33, 53-67, 68-75, 90-110, and 359-370 of SEQ ID NO:2 respectively), MEK1 (Accession Q01986) (residues 69-86, 107-120, 121-128, 141-153, and 382-393 of SEQ ID NO:4 respectively), JNK-3 (Accession BAA85877) (SEQ ID NOS:6, 10, 14, 18, and 22 respectively), GSK-3 (Accession 18158777) (SEQ ID NOS:7, 11, 15, 19, and 23 respectively), Akt (Accession P47197) (SEQ ID NOS:8, 12, 16, 20, and 24 respectively) and NIK (Accession T18359) (SEQ ID NOS:9, 13, 17, 21, and 25 respectively).

Please **amend** specification at paragraph 21 with the follow rewritten paragraph:

A3

[00021] (AMENDED) The following is a nonlimiting list of preferred chimeric protein kinases of the present invention: IKKβ/p38 ATP binding site chimera comprising an amino acid sequence of p38 with mutations Tyr 35 to Phe, Leu75 to Met, and Thr106 to Glu (e.g. SEQ ID NO:26); MEK1/p38 ATP binding site chimera comprising an amino acid sequence of p38 with mutations Tyr 35 to Gly and Thr106 to Glu (e.g. SEQ ID NO:27);

A3 cont.

JNK-3/p38 ATP binding site chimera comprising an amino acid sequence of p38 with mutations Tyr 35 to Gln, Leu75 to Met, and Thr106 to Met (e.g. SEQ ID NO:28); GSK-3/p38 ATP binding site chimera comprises of p38 with mutations Tyr 35 to Phe, Leu75 to Met, and Thr106 to Leu (e.g. SEQ ID NO:29); Akt/p38 ATP binding site chimera comprises p38 with mutations Tyr 35 to Phe and Thr106 to Glu (e.g. SEQ ID NO:30); NIK/p38 ATP binding site chimera comprises p38 with mutations Tyr 35 to Ala, Leu75 to Ser, and Thr106 to Val (e.g. SEQ ID NO:31); IKKβ/p38 inhibitor binding site chimera comprises p38 with mutations Lys79 to Asn, Glu81 to Pro, His 107 to Tyr, and C-terminus (351-356) PPLDQE to KPATQC (e.g. SEQ ID NO:32); MEK1/p38 inhibitor binding site chimera comprising an amino acid sequence of p38 with mutations Lys79 to Asn, Glu81 to Pro, and C-terminus (351-356) PPLDQE to THAASI (e.g. SEQ ID NO:33); JNK-3/p38 inhibitor binding site chimera comprising an amino acid sequence of p38 with mutations Lys79 to Asn, Glu81 to Lys, and His 107 to Glu (e.g. SEQ ID NO:34); GSK-3/p38 inhibitor binding site chimera comprising an amino acid sequence of p38 with mutations Lys79 to Asp, Glu81 to Cys, His 107 to Asp, and C-terminus (351-356) PPLDQE to PHARIQ (e.g. SEQ ID NO:35); Akt/p38 inhibitor binding site chimera comprises p38 with mutations Lys79 to Arg, Glu81 to Pro, His 107 to Tyr, and C-terminus (351-356) PPLDQE to FPQFSV (e.g. SEQ ID NO:36); NIK/p38 inhibitor binding site chimera comprising an amino acid sequence of p38 with mutations Lys79 to Arg, Glu81 to Val, His 107 to Asn, and C-terminus (351-356) PPLDQE to TLAVKE (e.g. SEQ ID NO:37).

Please **amend** specification at paragraph 23 with the follow rewritten paragraph:

A4

[00023] (AMENDED) In a further embodiment, the present invention is

Q4cont.

directed to a protein kinase inhibitor site which corresponds to an amino acid sequence of, and has three-dimensional structural homology to, a domain of p38 (SEQ ID NO:1) starting with the linker L5 (residues 78-85) that joins helix C (residues 63-77) with β4 (residues 84-89), the crossover connection (L7) (residues 100-115) and ending at the C-terminus (βL16) (residues 310-336). This domain has corresponding amino acids in IKK-β, Map/ERK kinase, eJNK, MEK, GSK-3, Akt, and NIK. *See* Figure 7. This inhibitor binding site is useful, *inter alia,* for designing protein kinase inhibitor molecules which do not bind to the ATP binding site of protein kinases. Therefore, the present invention also provides a method for identifying non-ATP binding site inhibitors of protein kinases comprising employing the structure of a protein kinase having an inhibitor bound to amino acids corresponding to amino acids Lys 79, Glu 81,His 107, Lys 165, and the C-terminus, 351-354 of p38, analyzing the three-dimensional structure of the protein kinase bound to the inhibitor, and designing an inhibitor of a protein kinase which also binds to amino acids corresponding to these amino acids of p38 by employing molecular modeling means.

Please **amend** specification at paragraph 50 with the follow rewritten paragraph:

Q5

[00050] (AMENDED) The inhibitor binding site of the present invention which is outside the ATP binding site comprises amino acids corresponding to a structural region of p38 (SEQ ID NO:1) wherein the region comprises linker L5 (residues 78 to 85) that joins helix C (residues 63-77) with β4 (residues 84-89), as well as the cross over connection, L7, (residues 100-115) and the C-terminus (βL16) (residues 310-336). The protein kinase inhibitor binding site of the present invention is at the hinge site between a helix rich domain of the

A5 cont.

protein kinase and a beta sheet rich domain of the protein kinase (referred to herein as the two domains of the protein kinase). The protein kinase inhibitor binding site of the present invention binds to, *inter alia*, the inhibitors sulindac sulfide {cis-5-flouro-2-methyl-1-[p-(methylsulfinyl)benzylidene]indene-3-acetic acid}(*see* Figure 2(a)), which inhibits cyclooxygenase and IKK-β and is a non-steroidal anti-inflammatory and PD98059 {1-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one} (*see* Figure 2(c)) which is a flavonoid which binds specifically to and inhibits the activation of MEK1 by c-Raf and other upstream activators [*see* Alessi et al., *J. Biol. chem.* 270:27489-27494 (1995)] and is non-competitive with respect to ATP hydrolysis [Dudley et al., *Proc. Natl Acad. Sci. USA* 92:7686-7689 (1995).

Please **amend** specification at paragraph 55 with the follow rewritten paragraph:

A6

[00055] (AMENDED) In a particularly preferred embodiment, the chimeric protein kinase of the present invention comprises nearly all the amino acids of p38 with just the amino acids of IKK-β corresponding to the inhibitor binding site of the present invention which is outside the ATP binding site. This chimeric protein kinase has the following mutations in the p38 amino acid sequence: His107 of p38 to Tyr, Glu81 of p38 to Pro, and Leu353 of p38 to Ala (e.g. SEQ ID NO:38). In another embodiment of the invention, the chimeric protein kinase of the present invention comprises nearly all the amino acids of p38 with just the amino acids of Map/ERK corresponding to the inhibitor binding site of the present invention and comprises the amino acid residues of p38 with the following mutations: Lys79 of p38 to Asn, Glu81 of p38 to Pro, and the sequence in the C-terminus of p38 (351-356) of PPLDQE to THAASI (SEQ ID NO:33). In a further embodiment of the invention, the chimeric protein

A4 cont.

kinase of the present invention comprises nearly all the amino acids of p38 with just the amino acids of JNK corresponding to the ATP binding and comprises the amino acid residues of p38 with the following mutations: Thr106 of p38 to Met, Tyr 35 of p38 to Gln, His107 of p38 to Glu and Leu75 of p38 to Met (e.g. SEQ ID NO:39).

Please **amend** specification at paragraph 57 with the follow rewritten paragraph:

A7

[00057] (AMENDED) The present invention is further directed to a method for identifying a protein kinase inhibitor which binds to the protein kinase inhibitor binding site of the present invention which is outside the ATP binding site. The method comprises crystallizing a protein kinase by methods known in the art; obtaining crystals comprising the protein kinase and PD98059 or sulindac sulfide (this may be accomplished by co-crystallizing the inhibitor PD98059 or sulindac sulfide with the protein kinase, or crystallizing the protein kinase alone and later soaking the crystals in a solution containing PD98059 or sulindac sulfide such that the PD98059 or sulindac sulfide enter the crystals and bind to the protein kinases), obtaining X-ray crystallographic data from the crystals comprising the protein kinase and PD98059 or sulindac sulfide by methods known in the art, determining the three-dimensional structure of the protein kinase and the PD98059 or sulindac sulfide from the X-ray crystallographic data, analyzing the three-dimensional structure of the P98509 or sulindac sulfide inhibitor binding site, designing an inhibitor compound which binds to the inhibitor binding site using molecular modeling means known in the art, and determining whether the designed inhibitor compound inhibits the protein kinase. In a preferred embodiment, the protein kinase is p38. In another preferred embodiment, the protein kinase is a chimeric protein kinase which

A7 cont.

comprises inhibitor binding site residues from a first protein kinase and non-inhibitor binding site residues from a second protein kinase. The first protein kinase may be non-crystallizable, or not easily crystallizable and the second protein kinase may be crystallizable. The resultant chimeric protein kinase is preferably crystallizable. In a further preferred embodiment, the chimeric protein kinase comprises amino acids Tyr98, Pro72, Ala 367 of IKK-β and the remainder from p38 (e.g. SEQ ID NO:38).

Please **amend** specification at paragraph 64 with the follow rewritten paragraph:

A8

[00064] (AMENDED) Binding site for sulindac sulfide and PD98059: The binding site in p38 for sulindac sulfide and PD98059 is at the hinge point between the two kinase domains. It is walled by the linker L5 (residues 78-85 of SEQ ID NO:1) that joins helix C (residues 63-77 of SEQ ID NO:1) with β4 (residues 84-89 of SEQ ID NO:1), the crossover connection (L7) (residues 100-115 of SEQ ID NO:1) and the C-terminus (βL16) (residues 310-336 of SEQ ID NO:1) (Figure 5). This site is outside the catalytic site Figure 4 shows the positions of the sulindac sulfide and PD98059 binding site of the present invention along with the the native ATP-competitive inhibitor binding site.

IN THE CLAIMS

Please **amend** claim 16 with the follow rewritten claim:

A9

16. (AMENDED) The chimeric protein kinase of claim 15 wherein p38 comprises amino acid changes of Lys79 to Asn, Glu81 to Pro, and the C-terminal sequence PPLDQE of p38 to THAASI.